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**PALMITIC AND OLEIC ACID: THE YIN AND YANG IN T2DM**  
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<b>Abstract:</b>	Increased plasma non-esterified fatty acids (FA) link obesity with insulin resistance (IR) and type 2 diabetes mellitus (T2DM). However, in contrast to the SFA acid palmitic acid, the MUFA oleic acid elicits beneficial effects on insulin sensitivity. Indeed, the dietary palmitic acid/oleic acid ratio impacts diabetic risk in humans. Here, we review the beneficial effects of oleic acid compared to palmitic acid on IR and T2DM, including its anti-inflammatory actions, and its capacity to inhibit endoplasmic reticulum stress, to prevent attenuation of the insulin signaling pathway and to improve beta-cell survival. Understanding the molecular mechanisms of the antidiabetic effects of oleic acid may contribute to understand the benefits of this FA in the prevention or delay of T2DM.

1 **PALMITIC AND OLEIC ACID: THE YIN AND YANG OF FATTY ACIDS IN**  
2 **TYPE 2 DIABETES MELLITUS**

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18 Inflammation.

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26 **Abstract**

27 Increased plasma non-esterified fatty acids link obesity with insulin resistance and type  
28 2 diabetes mellitus. However, in contrast to the saturated fatty acid palmitic acid, the  
29 monounsaturated fatty acid oleic acid elicits beneficial effects on insulin sensitivity.  
30 Indeed, the dietary palmitic acid/oleic acid ratio impacts diabetic risk in humans. Here,  
31 we review recent mechanistic insights into the beneficial effects of oleic acid compared  
32 to palmitic acid on insulin resistance and type 2 diabetes mellitus, including its anti-  
33 inflammatory actions, and its capacity to inhibit endoplasmic reticulum stress, to  
34 prevent attenuation of the insulin signaling pathway and to improve  $\beta$ -cell survival.  
35 Understanding the molecular mechanisms of the antidiabetic effects of oleic acid may  
36 contribute to understand the benefits of this fatty acid in the prevention or delay of type  
37 2 diabetes mellitus.

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51 **Increased plasma non-esterified fatty acids link obesity and insulin resistance**

52 Insulin resistance, defined as a defect in the capacity of insulin to drive glucose into its  
53 target tissues, both predicts and precedes the development of type 2 diabetes mellitus  
54 (T2DM) [1]. This condition eventually leads to hyperglycemia when pancreatic  $\beta$  cells  
55 fail to secrete sufficient insulin to meet the increased metabolic demand for this  
56 hormone. The expansion of adipose tissue in obese individuals releases increased  
57 amounts of non-esterified fatty acids (**NEFA**) (see Glossary, also called free fatty acids,  
58 FFA), hormones, pro-inflammatory cytokines and other factors that are involved in the  
59 development of insulin resistance. Increased levels of NEFA provoke insulin resistance  
60 and impair  $\beta$ -cell function [2], making them good candidates as a link between obesity  
61 and T2DM development. The link between obesity and T2DM also involves the  
62 activation of a chronic low-level inflammatory process promoted by increased levels of  
63 NEFA, which contributes to insulin resistance and T2DM [1].

64 Plasma NEFA released by adipose tissue reflects fat intake. Oleic acid (C18:1) and  
65 palmitic acid (C16:0) are the most abundant dietary and plasma FA, and represent 31%  
66 and 27% respectively of total plasma NEFA [3]. However, the saturated FA (SFA) (Box  
67 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differently contribute  
68 to insulin resistance. Studies of subjects who reduced their SFA and increased their  
69 MUFA intake have shown a significant improvement in insulin sensitivity [4]. This  
70 beneficial effect of oleic acid on insulin sensitivity can also help to explain the  
71 protective effects of the Mediterranean diet against both obesity and T2DM. That diet is  
72 characterized by a specific FA pattern with a low intake of SFA (7-8% of energy) and a  
73 high intake of MUFA (over 20% of total energy), as the primary fat source is olive oil  
74 (100 g contain 74 g of oleic acid) [5]. Understanding the molecular mechanisms

75 responsible for the beneficial effects of oleic acid might help promote its use in the  
76 prevention or delay of T2DM. Here, we review recent mechanistic insights into the  
77 beneficial effects of oleic acid compared to palmitic acid in insulin resistance and  
78 T2DM.

### 79 **Molecular mechanisms involved in palmitic acid-induced insulin resistance**

80 Three main mechanisms have been reported in palmitic acid-mediated insulin resistance  
81 and T2DM (**Figure 1**): 1) Increased synthesis of deleterious complex lipids; 2) Impaired  
82 function of cellular organelles; 3) Receptor-mediated inflammation. First, the increase  
83 in plasma NEFA favors the rate of FA delivery to non-adipose tissues, such as the liver,  
84 muscle, heart, and pancreatic islets. Such ectopic lipid deposition promotes  
85 metabolically relevant cellular dysfunction (lipotoxicity) and programmed cell-death  
86 (lipoapoptosis). In these circumstances, increased intracellular levels of palmitic acid  
87 exceed its mitochondrial oxidation and it is converted into deleterious complex FA-  
88 derived lipids, such as diacylglycerol (DAG) and ceramide. In contrast, the  
89 accumulation of the relatively inert triacylglycerol (TAG), which is not a signaling  
90 lipid, is generally considered to be harmless and it could even be protective [6]. The  
91 increase in the intracellular content of DAG caused by the exposure to enhanced levels  
92 of palmitic acid activates novel protein kinases C (PKC) isoforms, such as PKC $\theta$  in  
93 skeletal muscle and PKC $\epsilon$  in liver [7], which ultimately attenuate the insulin signaling  
94 pathway by phosphorylating serine residues in insulin receptor substrate 1 (IRS-1). The  
95 activation of some of these PKC isoforms, such as PKC $\theta$ , can also activate the pro-  
96 inflammatory signaling cascade I $\kappa$ B-kinase (IKK) $\beta$ -I $\kappa$ B-nuclear factor (NF)- $\kappa$ B [7]; a  
97 pathway which also attenuates insulin action through IKK $\beta$ -mediated phosphorylation

98 at serine residues of IRS-1 or by increasing the levels of pro-inflammatory cytokines.  
99 Interestingly, it has been reported that only DAG containing SFA in skeletal muscle  
100 membranes are related to insulin resistance in humans [8]. Likewise, the co-enzyme A  
101 thioester of palmitic acid is the limiting substrate of *de novo* ceramide synthesis.  
102 Therefore, the flux through this biosynthetic pathway is increased following enhanced  
103 availability of palmitic acid [9]. Increased ceramide synthesis leads to insulin resistance  
104 through several mechanisms, including inhibition of Akt via PKC $\zeta$  and protein  
105 phosphatase 2A (PP2A), which promotes dephosphorylation and inactivation of Akt [9].  
106 In addition, ceramide also induces endoplasmic reticulum (ER) stress, inhibits  
107 mitochondrial  $\beta$ -oxidation of FA and activates the nod-like receptor containing a pyrin  
108 domain (NLRP3) inflammasome [9]; a major instigator of inflammation.

109 Second, the mechanisms underlying the detrimental effects of excess NEFA on T2DM  
110 also include the impairment of the function of cellular organelles. Enhanced NEFA  
111 levels perturb ER homeostasis by causing lipid dysregulation in the ER that affects the  
112 lipid composition of the membranes of this organelle. This results in a process known as  
113 ER stress (Figure 1), which affects calcium signaling, can cause cell death, and  
114 attenuates protein translation [10]. In addition, palmitic acid-induced ER stress  
115 intersects with the inflammatory process by activating several pro-inflammatory  
116 pathways, such as NF- $\kappa$ B, c-Jun N-terminal kinase (JNK), double-stranded RNA  
117 (dsRNA)-dependent protein kinase (PKR) and the NLRP3 inflammasome [10].  
118 Ultimately, all these changes contribute to metabolic dysregulation and T2DM [10].

119 Mitochondrial dysfunction has also been associated with muscle insulin resistance in  
120 several studies [11]. T2DM is characterized by the presence of disturbances in FA

121 metabolism, and mitochondria dysfunction can result in a reduced oxidative capacity  
122 promoting intracellular accumulation of complex lipids that activate pathways that  
123 interfere with the insulin signaling pathway. In addition, it has been proposed that  
124 increased lipid oversupply into the mitochondria because of increased NEFA levels  
125 leads to incomplete FA oxidation provoking increased generation of reactive oxygen  
126 species (ROS), with concomitant mitochondrial stress, which ultimately leads to cellular  
127 damage and insulin resistance [12]. Moreover, FA translocase, or CD36, is a scavenger  
128 receptor that recognizes long-chain FA and is involved in their trafficking; it also plays  
129 an important role in T2DM. In fact, palmitic acid is a potent inducer of ROS in several  
130 cell types, including  $\beta$ -cells, and CD36 appears to be required for FA-induced ROS  
131 production [13].

132

133 Third, palmitic acid can activate pro-inflammatory pathways through membrane  
134 receptors, such as Toll-like receptor 4 (TLR4): a pattern recognition receptor that  
135 recognizes bacterial components, including lipopolysaccharide (LPS). SFA can also  
136 activate TLR4 through fetuin A: an adaptor protein that mediates the interaction  
137 between SFA and TLR4. Once activated, TLR4 subsequently promotes the activation of  
138 pro-inflammatory transcription factors such as NF- $\kappa$ B [14]. Moreover, SFA can modify  
139 gut microbiota leading to an increase in the levels of LPS after high fat intake,  
140 enhancing this natural TLR4 ligand [15]. In addition to TLR4, another molecule that  
141 integrates immune and nutritional signaling with inflammation and insulin action is  
142 PKR, which is activated by viral dsRNA as part of the mammalian immune response. It  
143 has been suggested that small nucleolar RNA (snoRNA), which accumulate in the

144 cytosol and act as critical mediators of lipotoxicity, interact and activate PKR in a  
145 palmitic acid-dependent manner [16,17].

146 As mentioned above, substituting palmitic acid by oleic acid, together with additional  
147 life style changes, offers an opportunity to reverse or delay the deleterious metabolic  
148 effects of this SFA. In fact, increased consumption of olive oil, which is high in oleic  
149 acid and contains antioxidant compounds, was associated with a reduction in the risk of  
150 T2DM in US women [18]. Likewise, it has been reported that the dietary palmitic  
151 acid/oleic acid ratio impacts diabetic risk in women [19]. Overall, these and other  
152 reports suggest that oleic acid has a protective effect against insulin resistance and  
153 T2DM. In the following sections, we describe the molecular mechanisms by which  
154 oleic acid prevents palmitic acid-induced insulin resistance and T2DM.

155

### 156 **Oleic acid-activated mechanisms that attenuate the increase in the synthesis of** 157 **deleterious complex lipids caused by palmitic acid**

158 Skeletal muscle is a key tissue that affects the metabolic state of the whole organism  
159 since it accounts for about 80% of insulin-stimulated glucose disposal and is the main  
160 organ for NEFA utilization [20]. Accumulation of deleterious complex lipids (DAG and  
161 ceramide) within muscle fibers is considered a key factor in the development of insulin  
162 resistance [1] and may occur because of increased FA uptake due to excess plasma  
163 NEFA and/or because of a reduced rate of mitochondrial FA oxidation [21]. We  
164 previously reported that exposure of myotubes to palmitic acid leads to enhanced DAG  
165 levels and the subsequent activation of the pro-inflammatory pathway PKC $\theta$ -NF- $\kappa$ B.  
166 This causes both phosphorylation of IRS-1 at serine residues and inhibition of insulin-

167 stimulated Akt phosphorylation, thereby attenuating insulin signaling [22] (Figure 2).  
168 However, exposure to oleic acid does not cause these changes and co-incubation of  
169 palmitic acid-exposed cells with a lower concentration of oleic acid reverses the effects  
170 of the latter. When we examined the incorporation of these FA into TAG and DAG, we  
171 observed that palmitic acid was mainly incorporated into DAG, whereas oleic acid was  
172 mainly incorporated into TAG. Furthermore, cells incubated with both palmitic and  
173 oleic acid showed a greater incorporation of FA into TAG than cells exposed only to  
174 palmitic acid. These findings have been confirmed in additional studies [23]. The  
175 increased channeling of palmitic acid into DAG was associated with a reduction in the  
176 expression levels of diacylglycerol acyltransferase-2 (*Dgat2*), which is the enzyme that  
177 catalyzes the conversion of DAG into TAG; whereas no changes were observed in cells  
178 incubated with either oleic acid or palmitic plus oleic acid. These differences might  
179 explain why palmitic acid increases the levels of DAG and activates the PKC $\theta$ -NF- $\kappa$ B  
180 pathway, whereas the addition of oleic acid favors the synthesis of TAG, protecting the  
181 cells from inflammation and the attenuation of the insulin signaling pathway. Moreover,  
182 palmitic acid and oleic acid affect the expression of genes involved in mitochondrial FA  
183 oxidation differently. The expression of these genes is regulated at the transcriptional  
184 level by several nuclear receptors, including PPAR $\alpha$  and PPAR $\beta/\delta$ , whose potency as  
185 transcription factors largely depends on the assembly of transcriptional co-activators,  
186 such as PPAR $\gamma$  co-activator (PGC)-1 $\alpha$ , which also controls mitochondrial biogenesis  
187 [24]. Palmitic acid reduces the expression of *Pgc-1 $\alpha$*  in myotubes, whereas oleic acid  
188 does not, and co-incubation of palmitic acid-exposed cells with lower concentration of  
189 oleic acid prevents the reduction in its expression. The capacity of oleic acid to prevent  
190 the downregulation in the expression of *Pgc-1 $\alpha$*  was dependent on PPAR $\alpha$  and protein

191 kinase A (PKA) [22]. PGC-1 $\alpha$  activity is also controlled through reversible acetylation.  
192 PGC-1 $\alpha$  is deacetylated and activated by sirtuin 1 (Sirt1), and acetylated and inhibited  
193 by general control of amino-acid synthesis 5-like 2 (GCN5). Interestingly, Sirt1  
194 deacetylase activity is induced by different signaling pathways, including AMPK and  
195 cyclic adenosine monophosphate (cAMP)/PKA, ultimately enhancing mitochondrial FA  
196 oxidation [25]. Importantly, myotubes exposed to oleic acid, but not to palmitic acid,  
197 display an increase in the levels of cAMP, which activate PKA. This results in SIRT1  
198 phosphorylation at serine 434, which increases its deacetylase activity, eventually  
199 leading to enhanced activity of PGC-1 $\alpha$  and expression of genes involved in  
200 mitochondrial FA oxidation, as well as the subsequent higher rate of FA oxidation [26].  
201 Oleic acid also increases the levels of carnitine palmitoyltransferase 1 (CPT-1) [22,27],  
202 which enables the transport of FA into mitochondria for  $\beta$ -oxidation. This effect of oleic  
203 acid results in a reduction of ceramide and DAG levels in palmitic acid-exposed cells  
204 [27]. Further, co-incubation with oleic acid increases mitochondrial  $\beta$ -oxidation of the  
205 FA through a second mechanism: activation of AMPK, which in turn inhibits acetyl-  
206 CoA carboxylase (ACC) and consequently its product malonyl-CoA, a CPT-1 inhibitor  
207 [26]. Similarly, oleic acid abolishes palmitic acid-induced ceramide synthesis in  
208 myotubes by preventing the upregulation of dihydroceramide desaturase 1: the enzyme  
209 responsible for generating ceramide from its precursor. This effect of oleic acid protects  
210 cells from attenuation of the insulin signaling pathway caused by the SFA [28].

211 In the liver, an excess of SFA, such as palmitic acid, induces lipotoxicity, leading to the  
212 development of non-alcoholic fatty liver disease (NAFLD), a process associated with  
213 insulin resistance. Moreover, the severity of NAFLD correlates with the presence of  
214 lipoapoptosis. In cultured hepatocytes, palmitic acid, but not oleic acid, impairs the

215 insulin signaling pathway [29]. Likewise, co-incubation with both FA increases the  
216 extent of steatosis, but oleic acid reduces apoptosis and the impairment of insulin  
217 signaling caused by palmitic acid. Moreover, palmitic acid-induced apoptosis  
218 suppresses autophagy by inducing caspase-dependent beclin 1 cleavage. In contrast, it  
219 was suggested that oleic acid protects hepatocytes against apoptosis by inducing the  
220 formation of TAG-enriched lipid droplets and induction of autophagy [30].

221 On the other hand, chronic exposure of rodent and human islet  $\beta$  cells to FA impairs  
222 glucose-stimulated insulin secretion (GSIS) and induces apoptosis through the  
223 activation of several pathways, including increased synthesis of ceramide,  
224 mitochondrial dysfunction, ER stress and activation of the long-chain FFA receptor 1  
225 (FFAR1) or G protein-coupled receptor 40 (GPR40) [31,32]. As for other cell types,  
226 oleic acid protects palmitic acid-exposed  $\beta$  cells, modifying the metabolism of the SFA  
227 either by increasing its oxidation or promoting its incorporation into TAG, thereby  
228 reducing the levels of ceramide and DAG, or by preventing ER stress [33-35]. The  
229 increase in palmitic acid-induced mitochondrial oxidation caused by oleic acid is  
230 mediated through PPAR $\beta/\delta$  activation [36].

231 **Oleic acid-activated mechanisms that prevent impaired function of cellular**  
232 **organelles caused by palmitic acid**

233 ER stress is a potentially new mechanism involved in the association between saturated  
234 NEFA-induced inflammation and insulin resistance [10]. We [37] and others [23] have  
235 reported that palmitic acid increases ER stress and inflammation and attenuates the  
236 insulin signaling pathway in mouse and human myotubes and *in vivo*. Oleic acid, in  
237 contrast, does not, and co-incubation of palmitic acid-exposed cells with oleic acid in a

238 lower concentration than the palmitic acid prevents these changes (Figure 3). This  
239 beneficial effect of oleic acid on palmitic acid-exposed cells is dependent on the AMP-  
240 mediated activation of AMPK [37], a metabolic sensor that also modulates  
241 inflammation and is recognized as a therapeutic target for diabetes [38]. The reduction  
242 in the levels of phosphorylated AMPK caused by palmitic acid in myotubes has also  
243 been associated with an increase in the levels of phosphorylated ribosomal protein S6  
244 kinase 1 (S6K1), a downstream kinase of mammalian target of rapamycin (mTOR) that  
245 attenuates the insulin signaling pathway by phosphorylating IRS-1 at serine residues  
246 [39]. Oleic acid does not affect the phosphorylation of S6K1, and co-incubation of  
247 palmitic acid-exposed myotubes with oleic acid prevents the increase caused by the  
248 SFA. In another study in human and mouse hepatocytes, oleic acid protected against the  
249 deleterious effects of palmitic acid on ER stress, apoptosis and the insulin signaling  
250 pathway by preventing S6K1 activation [40]. In a similar way, co-incubation of  
251 palmitic acid-exposed  $\beta$  cells with oleic acid protects the cells by activating pro-survival  
252 pathways of the ER stress response involved in protein folding and antioxidative  
253 defense [41].

254

255 Tribbles 3 (TRB3) is a pseudokinase, which contains a kinase domain without  
256 enzymatic activity and is responsible for strikingly different metabolic functions,  
257 depending on the tissue studied. In the liver, TRB3 binds to Akt and inhibits its activity,  
258 leading to impaired insulin signaling [42]. In hepatocytes, TRB3 upregulation occurs  
259 through palmitic acid-induced ER stress and the increased levels of this protein are  
260 sufficient to modulate FA-induced insulin resistance [43]. Oleic acid does not increase

261 TRB3 levels and co-incubation of palmitic acid-exposed cells with oleic acid prevents  
262 the increase in the levels of this protein.

263

264 Mitochondria are a major producer of ROS and exposure of skeletal muscle cells to  
265 palmitic acid causes a significant increase of mitochondrial ROS production and a  
266 concomitant mitochondrial DNA damage and dysfunction, induction of JNK, apoptosis,  
267 and inhibition of insulin signaling [44]. In contrast, supplementation with oleic acid  
268 reduces ROS generation and protects the mitochondria from palmitic acid-induced  
269 oxidative stress, apoptosis and attenuation of insulin signaling [44,45].

270

#### 271 **Oleic acid-mediated mechanisms that prevent palmitic acid-induced inflammation**

272 IL-1 $\beta$  is a potent cytokine that plays a key role in inflammation and insulin resistance  
273 [46]. In fact, lack of IL-1 $\beta$  signaling results in improved insulin sensitivity in HFD-fed  
274 mice [47]. The release of this potent cytokine requires two steps (Figure 4). First,  
275 activation of the TLR4/NF- $\kappa$ B pathway by palmitic acid leads to the production of pro-  
276 IL-1 $\beta$ . Second, the NLRP3 inflammasome generates mature IL-1 $\beta$  through caspase-1-  
277 dependent processing activated by several molecules, including ATP. It should be noted  
278 that substitution of SFA by an oleic acid-enriched diet in HFD-fed mice results in  
279 improved insulin sensitivity, reduced pro-IL-1 $\beta$  levels and adipose IL-1 $\beta$  secretion and  
280 sustained adipose AMPK activation [48]. Furthermore, oleic acid prevents ATP-induced  
281 IL-1 $\beta$  secretion from LPS- and palmitic acid-exposed macrophages through an AMPK-  
282 dependent mechanism. The findings of this study indicate that sustained activation of

283 AMPK by oleic acid disrupts NLRP3 inflammasome activation, reducing the processing  
284 of IL-1 $\beta$  and the attenuation of insulin signaling caused by this cytokine.

285 Several factors can increase the diabetogenic effect of visceral adipose tissue, including  
286 increased production of adipokines, which can impair insulin sensitivity. This  
287 production of cytokines is enhanced by the infiltration of macrophages into visceral  
288 adipose tissue [49]. Macrophages undergo specific differentiation depending on the  
289 local tissue environment, to fulfill their functionally distinct roles, and they are capable  
290 of polarizing toward different phenotypes, which include the classical (pro-  
291 inflammatory, M1) and alternative (anti-inflammatory, M2) activation states. In obesity,  
292 adipose tissue M1 macrophage numbers increase and correlate with adipose tissue  
293 inflammation and insulin resistance; whereas in lean humans and mice, M2  
294 macrophages predominate [50].

295 The effects of different FA have been evaluated in mature visceral adipocytes from non-  
296 obese and morbidly obese subjects [51]. Palmitic acid shows a direct inflammatory  
297 effect in adipocytes from non-obese subjects, increasing the expression of the pro-  
298 inflammatory cytokines TNF- $\alpha$  and IL-6 and decreasing the mRNA levels of the anti-  
299 inflammatory cytokine IL-10 and adiponectin: the most abundant peptide secreted by  
300 adipocytes, reduction of which plays a central role in insulin resistance/T2DM. These  
301 pro-inflammatory effects of palmitic acid are more pronounced in adipocytes from  
302 morbidly obese patients. In contrast, oleic acid decreases the expression of pro-  
303 inflammatory cytokines and causes a pronounced increase in IL-10 and adiponectin  
304 expression in adipocytes from non-obese patients (Figure 4). Interestingly, adiponectin  
305 increases cellular ceramidase activity, which catalyzes ceramide degradation and  
306 activates AMPK, which in turn can activate FA oxidation [52]. The anti-inflammatory

307 effect of oleic acid was abolished by knocking down the expression of FFAR4/GPR120,  
308 a receptor with anti-inflammatory effects that is activated by long-chain FA, mainly n-3  
309 PUFA, but also by oleic acid [53]. It is noteworthy that the pro-inflammatory effect of  
310 palmitic acid was also reversed by silencing FFAR4/GPR120, although no explanation  
311 was provided for this finding. Interestingly, the anti-inflammatory effect of oleic acid  
312 was not observed in adipocytes from morbidly obese subjects [51]. This might be a  
313 consequence of the reported reduced expression of FFAR4/GPR120 in visceral adipose  
314 tissue of morbidly obese patients [54]. Consistent with FFAR4/GPR120 playing a role  
315 in the mediation of the effects of oleic acid, an oleic acid-enriched diet improved whole-  
316 body insulin resistance in an animal model of diet-induced obesity by: reducing  
317 inflammation in adipose tissue, liver and skeletal muscle; decreasing macrophage  
318 infiltration; and increasing IL-10 levels, and all these effects were abolished by  
319 FFAR4/GPR120 knockdown [55]. Additionally, oleic acid increases M2 macrophage  
320 markers in mesenteric adipose tissue, suggesting that this MUFA induces M2  
321 macrophage polarization [56].

322 Oleic and palmitic acid also elicit opposite effects on macrophages/Kupffer cells and  
323 hepatocytes [57]. In fact, a conditioned medium from macrophages stimulated with  
324 palmitic acid induces ER stress and inflammatory signaling that impairs insulin  
325 signaling in mouse hepatocytes. In contrast, oleic acid-stimulated macrophages result in  
326 M2 polarization and the conditioned medium from these cells enhances insulin  
327 sensitivity in hepatocytes. This is due to a reduction in the levels of leukotriene B<sub>4</sub>,  
328 which in turn leads to a reduction in hepatocytes of both protein tyrosine phosphatase  
329 1B (PTP1B), and phosphatase and tensin homolog (PTEN): two phosphatases that  
330 negatively modulate insulin receptors and Akt, respectively. Oleic acid also upregulates

331 microRNA-21, inducing PTEN degradation; whereas palmitic acid has no effect [58].  
332 Moreover, oleic acid increases the expression of PPAR $\beta/\delta$  in human hepatocytes via a  
333 FFAR1/GPR40-mediated and calcium-dependent mechanism. The increase in PPAR $\beta/\delta$   
334 caused by oleic acid then negatively regulates PTEN and thereby increases insulin  
335 sensitivity [59].

336 Attenuation of insulin-induced tyrosine phosphorylation and methylation of the catalytic  
337 subunit of PP2A are involved in the activation of this enzyme by palmitic acid, and  
338 ultimately result in the repression of Akt activation/phosphorylation. In rat and human  
339 skeletal myotubes the effect of palmitic acid on PP2A is antagonized by oleic acid  
340 through increased tyrosine phosphorylation and demethylation of the catalytic subunit  
341 of PP2A [60], which results in the repression of the phosphatase activity of this enzyme.

342

#### 343 **Additional antidiabetic effects of oleic acid**

344 Part of the effects resulting from dietary intake of oleic acid may be mediated by its  
345 derived endogenous lipid mediator oleoylethanolamide (OEA). Dietary intake of oleic  
346 acid elevates the levels of OEA by increasing substrate availability for its biosynthesis  
347 from membrane glycerophospholipids [61]. Interestingly, OEA decreases food intake  
348 and body weight gain through peroxisome proliferator-activated receptor (PPAR) $\alpha$   
349 activation. The reduction in body weight caused by OEA has been attributed to its  
350 anorectic activity as well as to its capacity to increase lipolysis in adipocytes and the  
351 subsequent stimulation of FA oxidation in skeletal muscle [61]. Additionally, oleic acid  
352 ingestion stimulates OEA mobilization into the mucosal cells of the gut, which activates

353 a PPAR $\alpha$ -mediated signal that travels through the afferent vagus nerve to the  
354 hypothalamus, where it increases satiety [62].

355 On the other hand, dietary fat is known to stimulate the release by intestinal L-cells of  
356 glucagon-like peptide 1 (GLP-1), which in turn increases GSIS, among other  
357 physiological effects. The release of GLP-1 by dietary fat is mediated by NEFA through  
358 FFAR1/GPR40. In addition, oleic acid can also induce GLP-1 secretion *in vivo* via  
359 PKC $\zeta$  [63]. Meanwhile, GPR119 is an antidiabetic target expressed in  $\beta$  cells and in  
360 incretin-secreting enteroendocrine cells in the intestine [64]. Activation of GPR119 can  
361 increase insulin secretion directly by acting at the level of the  $\beta$ -cell or indirectly by  
362 increasing circulating levels of GLP-1 released from intestinal L-cells. Of all the  
363 endogenous GPR119 agonists, OEA is the more potent [64]. There are three additional  
364 oleic acid derivatives that are GPR119 agonists [64]. However, there are conflicting  
365 reports regarding whether these compounds are responsible for fat-induced GPR119  
366 activation and GLP-1 release [64]. OEA can also elicit a powerful cytoprotective effect  
367 on palmitic acid-exposed rat  $\beta$  cells. However, this effect is not mediated by GPR119,  
368 since OEA was internalized and subjected to hydrolysis by FA amide hydrolase to  
369 release free oleic acid, which in turn mediated cytoprotection [65].

370

371 Some of the effects of oleic acid might also be mediated by its effects on the  
372 hypothalamus, a region that regulates energy homeostasis. The hypothalamus matches  
373 energy intake to energy expenditure to prevent obesity through satiety (leptin) and  
374 hormonal (insulin) signals. In addition, the hypothalamus interacts with the liver  
375 through the vagus nerve. On sensing FA, the hypothalamus lowers food intake and

376 glucose production [66]. For instance, central oleic infusion inhibits glucose production  
377 and food intake [67]. Exposure to palmitic acid, but not oleic acid, by oral gavage or  
378 direct infusion results in PKC $\theta$  activation in the hypothalamus, which was associated  
379 with impaired hypothalamic insulin and leptin signaling [68]. In humans, increased  
380 levels of oleic acid in cerebrospinal fluid were associated with improved glucose  
381 tolerance [69].

382 Cardiovascular disease is a major cause of morbidity and mortality in T2DM patients,  
383 and the presence in these patients of atherogenic dyslipidemia is among the major  
384 factors contributing to increased cardiovascular risk. It is now generally accepted that  
385 the different components of atherogenic dyslipidemia are closely linked and are initiated  
386 by insulin resistance through overproduction of TAG-rich very-low-density lipoproteins  
387 (VLDL) [70]. Interestingly, infusion of oleic acid in the hypothalamus of rats activates a  
388 neuronal network that suppresses VLDL-TAG secretion in the liver [71]. Oleic acid also  
389 shows a beneficial effect at the cardiovascular level, compared with palmitic acid [72].

### 390 **Concluding remarks and future perspectives**

391 There is compelling evidence that insulin resistance and T2DM can be prevented or  
392 their onset delayed by lifestyle interventions; and it is critical to promote changes in diet  
393 that reduce the incidence of T2DM [73]. It is now well accepted that the total amount of  
394 fat intake has an impact on insulin sensitivity only if it exceeds the 37% of total energy  
395 intake [4]. However, below this value, a critical factor in the induction of insulin  
396 resistance is not the total amount of fat, but its FA composition [4]. In fact, insights  
397 gained from preclinical studies have shown that substitution of part of the palmitic acid  
398 by oleic acid strongly attenuates the deleterious effects of the SFA on adipose tissue,

399 skeletal muscle, liver and  $\beta$  cells. Consistent with this, substituting dietary SFA for a  
400 high-oleic-acid diet enhances insulin sensitivity in humans [4]. Several mechanisms  
401 account for the protective effects of oleic acid with respect to T2DM. However, the  
402 studies have some limitations and many questions remain regarding the effects of oleic  
403 acid on humans (see Outstanding Questions). Interestingly, oleic acid activates or  
404 prevents the reduction in AMPK activity caused by palmitic acid. AMPK is a  
405 recognized therapeutic target for T2DM [38] and it is also the main target activated by  
406 metformin, the most commonly prescribed drug for T2DM in the world [38]. Some  
407 studies have demonstrated that oleic acid and metformin have similar protective effects,  
408 both preventing the deleterious effects of palmitic acid [39]; this suggests that oleic acid  
409 might have some metformin-like effects. Current and future studies should elucidate the  
410 mechanisms responsible for the protective effects of oleic acid against T2DM  
411 development in humans.

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623

624

625 **FIGURE LEGENDS**

626 **Figure 1. Palmitic acid attenuates the insulin signaling pathway through different**  
627 **mechanisms leading to insulin resistance.**

628 As visceral obesity develops, it results in greater release of NEFA, since visceral  
629 adipocytes are more sensitive to lipolytic stimuli. In addition, insulin resistance leads to  
630 the failure of lipolysis inhibition by this hormone and further augments the increase in  
631 plasma NEFA levels. The increase in plasma NEFA, mainly the saturated palmitic acid,  
632 results in inflammation and insulin resistance via three main mechanisms. 1. Increased  
633 internalization of palmitic acid results in lipotoxicity when this FA exceeds oxidative  
634 needs and spills over into deleterious non-oxidative metabolic pathways, increasing the  
635 levels of diacylglycerol (DAG) and ceramide. DAG activates PKC, which in turn  
636 attenuates the insulin signaling pathway by phosphorylating IRS-1 at serine residues  
637 and it also activates the IKK $\beta$ -NF- $\kappa$ B pathway, further exacerbating the impairment of  
638 insulin signaling and provoking inflammation. The increase in ceramide activates the  
639 NLRP3 inflammasome-mediated release of IL-1 $\beta$ , and it also leads to activation of  
640 PP2A and PKC, which both attenuate the insulin signaling pathway. 2. The excess of  
641 palmitic acid impairs the function of the ER and mitochondria. Impairment of ER  
642 homeostasis results in ER stress that promotes inflammation (NF- $\kappa$ B, JNK and NLRP3  
643 inflammasome pathways are activated) and apoptosis (e.g.  $\beta$  cells). The impairment of  
644 mitochondrial function may reduce FA oxidation and it can increase ROS generation. 3.  
645 Palmitic acid can activate TLR-4 through fetuin A and high-fat diets increase the levels  
646 of LPS, an activator of this receptor, leading to an increase in the activity of the IKK $\beta$ -  
647 NF- $\kappa$ B pathway.

648 Akt: mammalian homologue of retroviral oncogene v or protein kinase B; DAG:  
649 diacylglycerol; ER: endoplasmic reticulum; FA: fatty acids; FAT: fatty acid translocase;  
650 FATP: fatty acid transporter protein; IL: interleukin; I $\kappa$ B: inhibitor of  $\kappa$ B; IKK $\beta$ :  
651 inhibitor of nuclear factor (NF)- $\kappa$ B kinase subunit  $\beta$ ; IRS: insulin receptor substrate;  
652 JNK: c-Jun N-terminal kinase; LPS: lipopolysaccharide; NLRP3: nod-like receptor  
653 containing a pyrin domain; NF- $\kappa$ B: nuclear factor- $\kappa$ B; PI3K: phosphatidylinositol 3  
654 kinase; PKC: protein kinase C; pS: phosphorylated serine; PP2A: protein phosphatase  
655 2A; pY: phosphorylated tyrosine; p50/p65: NF- $\kappa$ B subunits; ROS: reactive oxygen  
656 species; TAG: triacylglycerol; TLR4, Toll-like receptor 4.

657

658

659 **Figure 2. Oleic acid prevents the palmitic acid-induced increase in the synthesis of**  
660 **deleterious complex lipids.** Oleic acid prevents the deleterious effects of palmitic acid  
661 by increasing the mitochondrial oxidation of the SFA and by promoting their  
662 accumulation in the form of TAG, thereby reducing the synthesis of DAG and  
663 ceramide. The increase in FA oxidation caused by oleic acid is mediated by the  
664 restoration of AMPK activity and the increase in the expression of *Cpt-1* and *Pgc-1 $\alpha$* . In  
665 addition, oleic acid increases the activity of PGC-1 $\alpha$  through the activation of Sirt1 and  
666 the subsequent reduction of the acetylated (inactive) form of this protein. Oleic acid  
667 prevents the reduction in AMPK activity caused by palmitic acid and ultimately inhibits  
668 ER stress and inflammation as well as the activation of the mTORC1-S6K1 pathway.  
669 Blue arrows show the potential effects of oleic acid.

670

671 Ac: acetylated; Akt: mammalian homologue of retroviral oncogene v or protein kinase  
672 B; AMPK: AMP-activated protein kinase; CPT-1: carnitine palmitoyl transferase 1;  
673 DAG: diacylglycerol; DAGT2: diacylglycerol acyltransferase 2; FA: fatty acids; FAT:  
674 fatty acid translocase; FATP: fatty acid transporter protein; I $\kappa$ B: inhibitor of  $\kappa$  B; IKK $\beta$ :  
675 inhibitor of nuclear factor (NF) $\kappa$ -B kinase subunit  $\beta$ ; IL: interleukin; IRS: insulin  
676 receptor substrate; NF- $\kappa$ B: nuclear factor- $\kappa$ B; p50/p65: NF- $\kappa$ B subunits; PGC-1 $\alpha$ :  
677 PPAR $\gamma$  co-activator 1 $\alpha$ ; PI3K: phosphatidylinositol 3 kinase; PKC: protein kinase C;  
678 PPAR: peroxisome proliferator-activated receptor; pS: phosphorylated serine; PP2A:  
679 protein phosphatase 2A; pY: phosphorylated tyrosine; p50/p65: NF- $\kappa$ B subunits; ROS:  
680 reactive oxygen species; RXR: retinoid X receptor; SFA, saturated fatty acids; TAG:  
681 triacylglycerol.

682

683

684

685 **Figure 3. Oleic acid prevents the impairment in the function of cellular organelles**  
686 **caused by palmitic acid.** Oleic acid prevents the reduction in AMPK activity caused by  
687 palmitic acid and ultimately inhibits ER stress and inflammation as well as the  
688 activation of the mTORC1-S6K1 pathway. Oleic acid also prevents the increase in the  
689 levels of the ER stress-induced pseudokinase TRB3 caused by palmitic acid. Moreover,  
690 oleic acid attenuates ROS generation and protects the mitochondria from palmitic acid-  
691 induced oxidative stress.

692 Akt: mammalian homologue of retroviral oncogene v or protein kinase B; AMPK:  
693 AMP-activated protein kinase; ER, endoplasmic reticulum; FAT: fatty acid translocase;  
694 FATP: fatty acid transporter protein; I $\kappa$ B: inhibitor of  $\kappa$  B; IKK $\beta$ : inhibitor of nuclear

695 factor (NF) $\kappa$ -B kinase subunit  $\beta$ ; IL: interleukin; IRS: insulin receptor substrate;  
696 mTOR: mammalian target of rapamycin; NF- $\kappa$ B: nuclear factor- $\kappa$ B; p50/p65: NF- $\kappa$ B  
697 subunits; PI3K: phosphatidylinositol 3 kinase; pS: phosphorylated serine; pY:  
698 phosphorylated tyrosine; p50/p65: NF- $\kappa$ B subunits; ROS: reactive oxygen species;  
699 TNF- $\alpha$ : Tumour necrosis factor  $\alpha$ .

700

701

702

703 **Figure 4. Oleic acid prevents palmitic acid-induced inflammation.**

704 Oleic acid elicits anti-inflammatory effects by reducing the levels of cytokines (IL-6 and  
705 TNF- $\alpha$ ), while increasing the levels of the anti-inflammatory cytokine IL-10 and  
706 adiponectin. These effects of oleic acid might be mediated by FFAR4/GPR120. The  
707 increase in the levels of adiponectin caused by oleic acid can activate AMPK and reduce  
708 the levels of palmitic acid-induced ceramide synthesis. By preventing the reduction in  
709 AMPK caused by palmitic acid, oleic acid can inhibit the increase in IL-1 $\beta$  caused the  
710 SFA through NLRP3 inflammasome in adipocytes and macrophages that infiltrate  
711 adipose tissue. Oleic acid might also favor the polarization of adipose tissue-infiltrated  
712 macrophages to the M2 anti-inflammatory phenotype. Oleic acid induces polarization of  
713 macrophages to the M2 anti-inflammatory phenotype, reducing the secretion of LTB<sub>4</sub>,  
714 which in turn decreases the activity of the phosphatases PTEN and PTP1B, thereby  
715 enhancing insulin sensitivity.

716

717 Akt: mammalian homologue of retroviral oncogene v or protein kinase B; AMPK,  
718 AMP-activated protein kinase; IL: interleukin; FA: fatty acids; GPR120/FFAR4: G-

719 protein-coupled receptor 120/free fatty acid receptor 1; IRS: insulin receptor substrate;  
720 LTB4: leukotriene B4; NF- $\kappa$ B: nuclear factor- $\kappa$ B; NLRP3: nod-like receptor containing  
721 a pyrin domain; PIP<sub>2</sub>: phosphatidylinositol 2-phosphate; PIP<sub>3</sub>: phosphatidylinositol 3-  
722 phosphate; PI3K: phosphatidylinositol 3 kinase; PTEN: phosphatase and tensin  
723 homolog; PTP1B: protein tyrosine phosphatase 1B; pY: phosphorylated tyrosine;  
724 p50/p65: NF- $\kappa$ B subunits; SFA, saturated fatty acid; TLR4, toll-like receptor 4.

725

726

727 **BOX 1: Fatty acids and the oleic acid-derivative oleoylethanolamide.**

728 It is recommended that dietary fat constitute 20-35% of energy intake [74]. However,  
729 the type of FA present in the fat ingested is of considerable importance in the  
730 development of insulin resistance/T2DM. FA can be classified according to their chain  
731 length into: short-chain FA, with 2-6 carbon atoms; medium-chain FA, with 7-12  
732 carbon atoms; long-chain FA, with 13-22 carbon atoms; and very-long-chain FA, with  
733 more than 22 carbon atoms. They are also divided into saturated FA (SFA), with no  
734 carbon-carbon double bonds; monounsaturated FA (MUFA), with a single double bond  
735 (alkene) in the FA chain; and polyunsaturated FA (PUFA), containing several double  
736 bounds. PUFA are subdivided into omega-6 (n-6) and omega-3 (n-3) acids depending  
737 on the distance from the first double bond to the methyl end. The ingestion of oleic acid  
738 can stimulate the production of oleoylethanolamide (OEA) in enterocytes [62].  
739 However, excess fat can reduce the synthesis of OEA. OEA belongs to the group of FA  
740 called ethanolamides or *N*-acylethanolamines, which are a class of naturally occurring  
741 bioactive signaling lipids derived from SFA and unsaturated FA. OEA controls food  
742 intake and promotes fat catabolism. Fat ingestion results in the release of oleic acid in  
743 the small-intestinal lumen, which is internalized by the enterocytes and channeled

744 toward the synthesis of chylomicrons or OEA. The latter can then be hydrolyzed into

745 oleic acid and ethanolamine by FA amide hydrolase (FAAH).

746

1 **Trends**

2 Substituting saturated fatty acids by oleic acid in the diet improves insulin sensitivity in  
3 humans.

4

5 Preclinical studies have shed light on the molecular mechanisms by which oleic acid  
6 prevents palmitic acid-induced inflammation and insulin resistance in adipose tissue,  
7 liver, skeletal muscle, and pancreas.

8

9 The hypothalamus also senses oleic acid, where it activates a neuronal network that  
10 suppresses VLDL-TAG secretion in liver.

11

12 Oleic acid protects against cardiovascular insulin resistance and the atherosclerotic  
13 process.

14

15 Some of the effects of oleic acid are mediated by preventing the reduction in palmitic  
16 acid-mediated AMPK activity, resembling the action of metformin.

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1 **Outstanding Questions**

2 Which oleic acid-activated molecular mechanism is the most important for preventing  
3 palmitic acid-induced insulin resistance?

4

5 Are the molecular mechanisms activated by oleic acid reported in preclinical models  
6 also observed in humans?

7

8 How much does OEA contribute to the effects of oleic acid? Are the effects of OEA on  
9 the release of GLP-1 mediated by GPR119 activation or are other molecular  
10 mechanisms involved?

11

12 Could an oleic acid-enriched diet be considered a non-pharmacological treatment to  
13 increase AMPK activity in humans, especially in those populations with a low  
14 consumption of this MUFA?

15

16 What is the contribution of oleic acid consumption to the beneficial effects of the  
17 Mediterranean diet on the prevention of insulin resistance and T2DM?

18

19

20

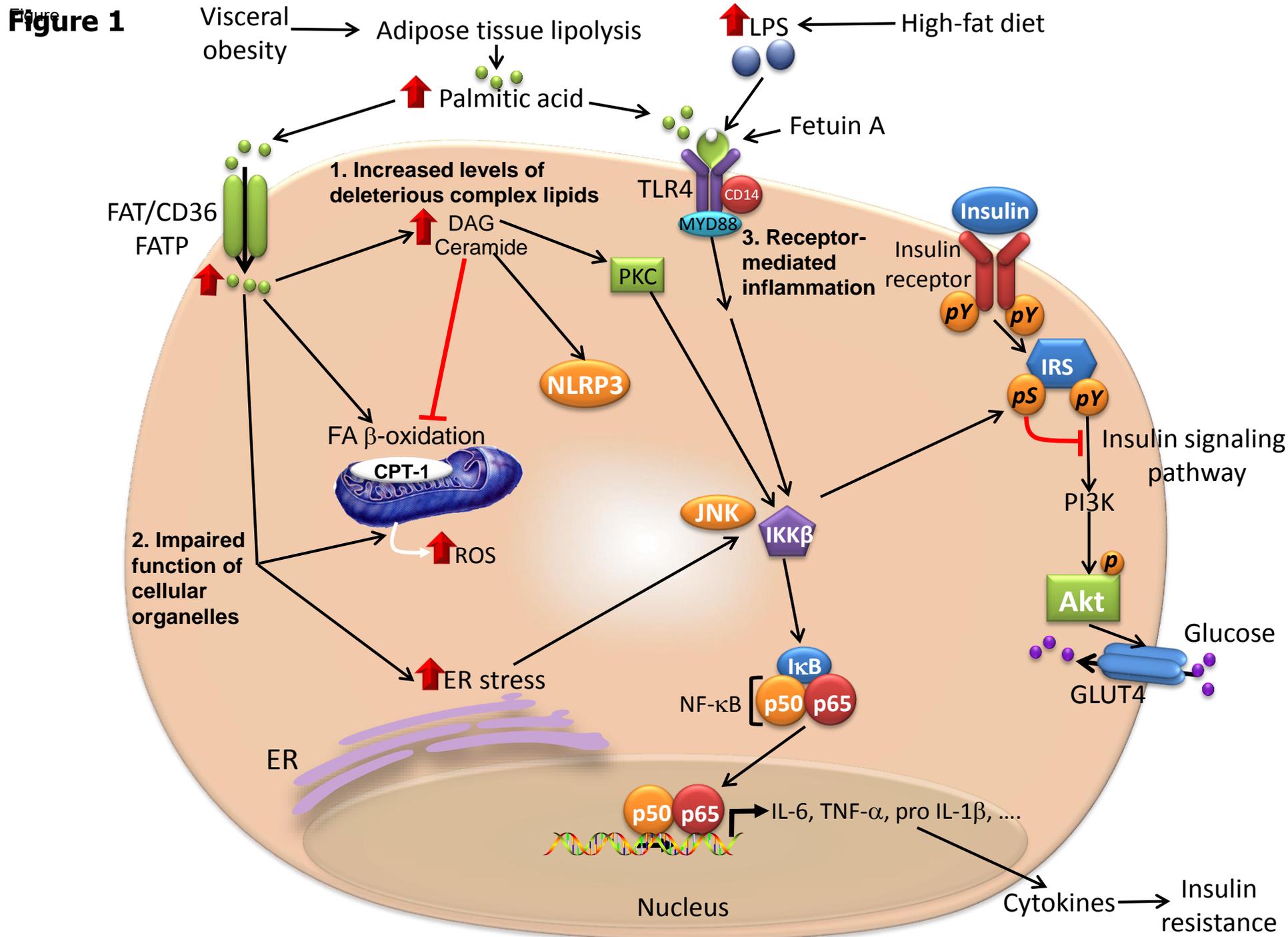
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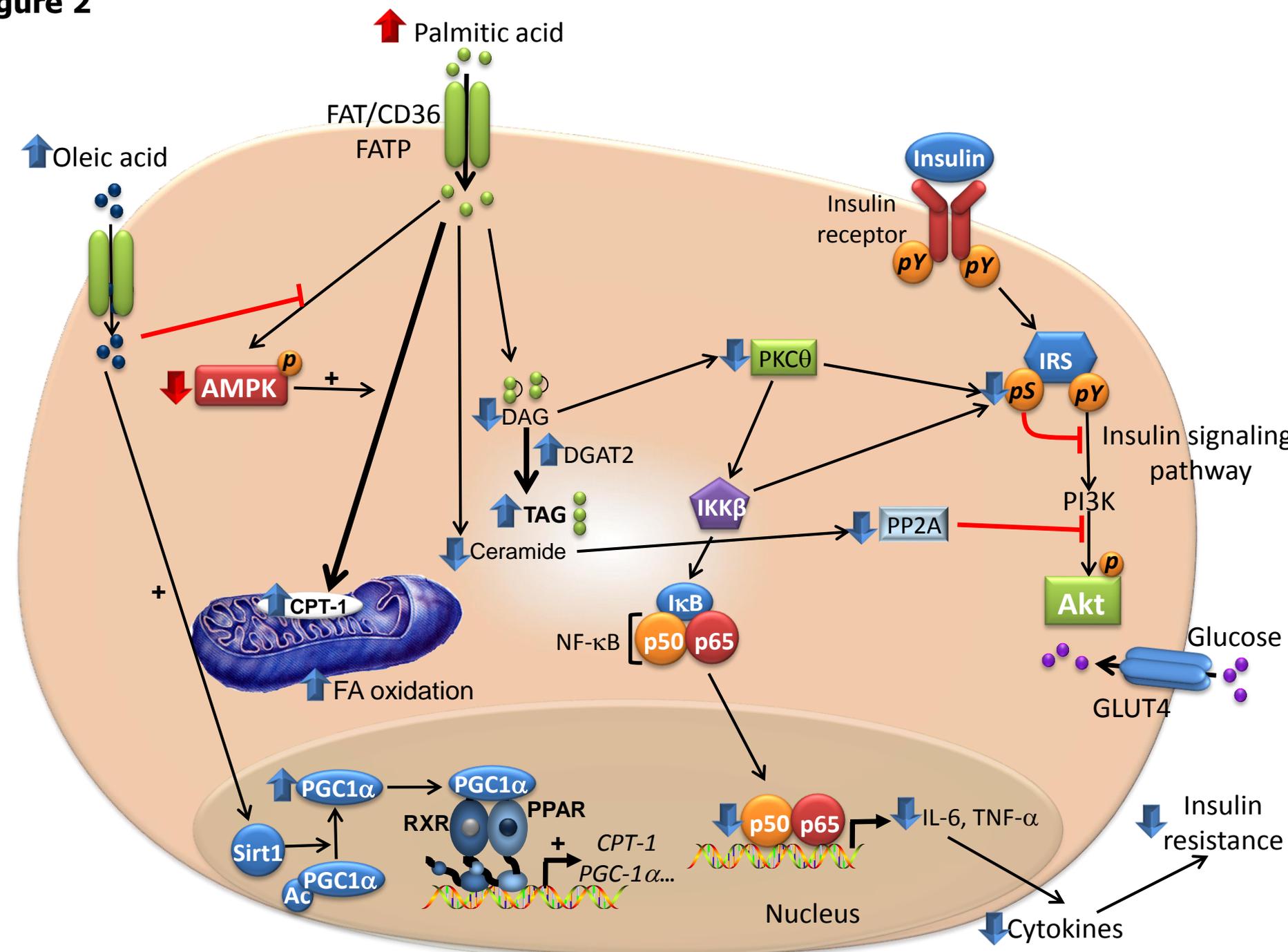
23

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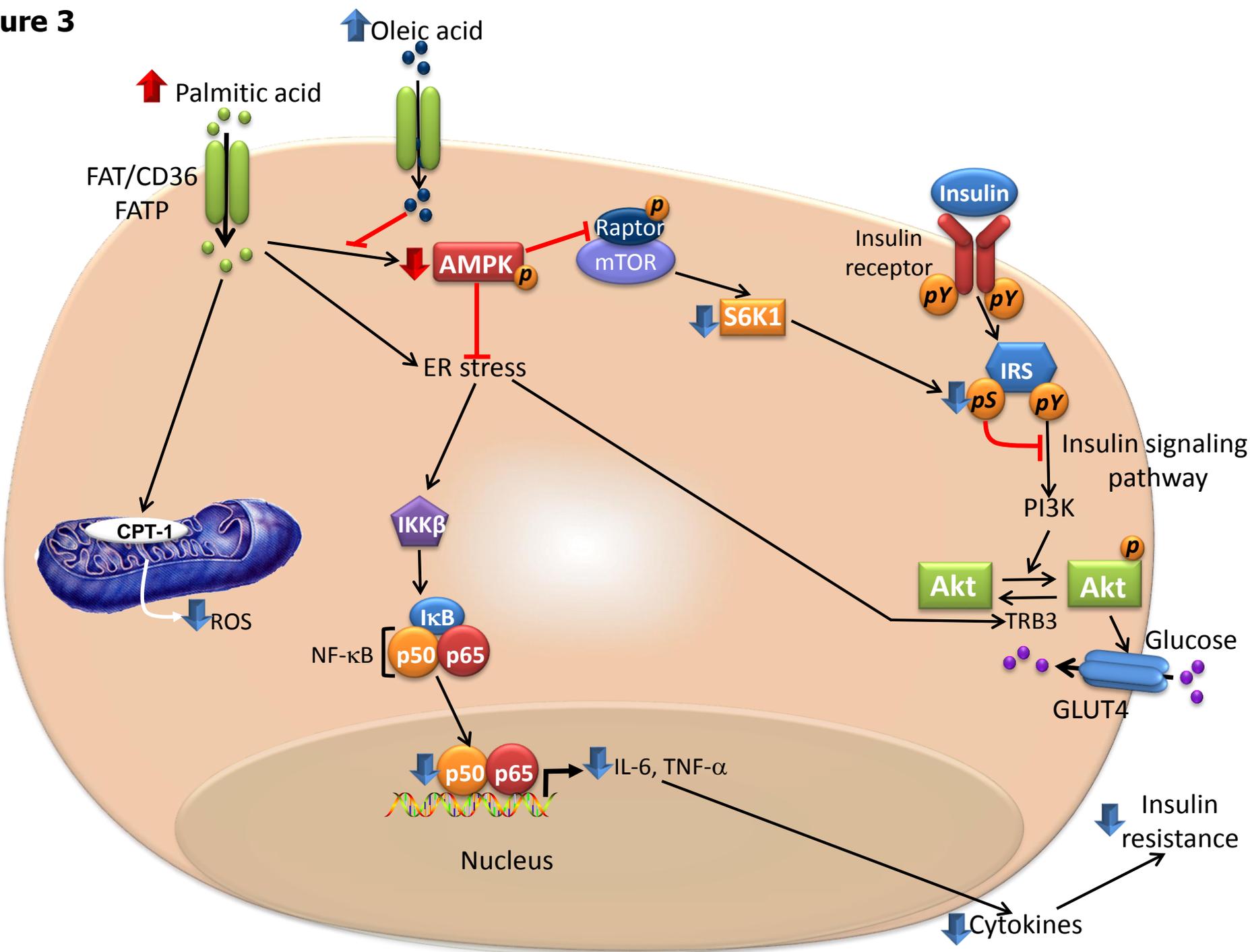
25



**Figure 2**

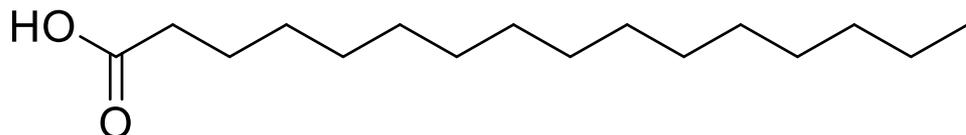


**Figure 3**

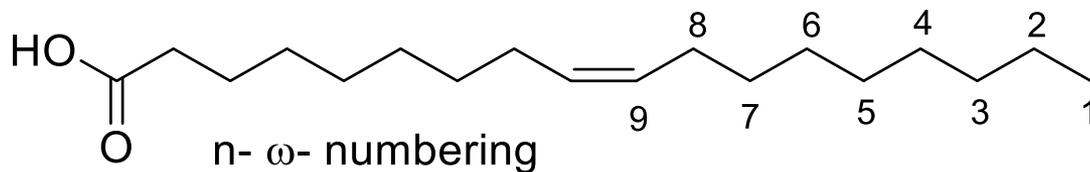




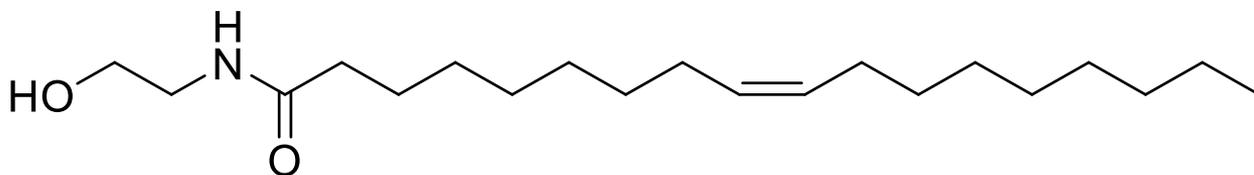
**Figure I**



Palmitic acid (C16:0)



Oleic acid (C18:1 n-9)



Oeloylethanolamide (OEA)



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November 22th, 2017

Matthew BEYMER  
Editor, Trends in Endocrinology & Metabolism

**TEM-D-17-00153**

Dear Dr. Beymer,

According to your instructions we wish to submit a reworked version of our manuscript entitled "PALMITIC AND OLEIC ACID: THE YIN AND YANG IN T2DM".

We submit a letter where we have addressed, point-by-point, all the issues raised by the reviewers. I thank you in advance for your interest in our work.

Yours truly,

Manuel Vázquez-Carrera

## Editorial Comments

We are very grateful to the comments of the Editor (*A nice timely review focusing the opposing effects of palmitate and oleate*).

### **Comment 1. INTRODUCTION**

*(increase plasma...)* Nice however rather lengthy I would limit the explanation of T2D and focus on setting up background on Oleic and Palmitic acids (see marked up manuscript).

According to the editor suggestion, the length of the introduction section has been reduced.

**Comment 2.** *(molecular mechs involved in palmitic....)* A very nice thorough and detailed explanation of the molecular mechanisms of palmitate-induced Insulin Resistance. Currently, I would say this section is a bit too long however if you focus and edit the following sections as suggested I would say it is sufficient.

To reduce the length of the manuscript, we have deleted a paragraph in this section referring to the athlete paradox.

### **Comment 3. CONTENT/ STRUCTURE/WORDING AND ACCESSIBILITY**

*As noted by Reviewer 2 I believe that the manuscript can be greatly improved with some re-organization. It does seem that through the review you are discussing tissue-specific effects of palmitate and oleate. Given the length of the current manuscript perhaps it would be prudent to keep examples from each tissue as evidence for the actions of oleate or palmitate and move tissue-specific actions to text boxes instead if this is something you are in fact trying to highlight, this would, however, require the elimination of the current figures, but from an editorial perspective I believe the 4 text boxes (adipose, skeletal, hepatic, and beta-cell specific actions) and on solid figure on palmitate actions and one solid figure on basic oleate actions would be very beneficial to the reader.*

We really appreciate the efforts of the editor and reviewer 2 to improve the manuscript. We have tried to follow this suggestion, but we found that describing the general mechanisms by which oleic acid prevents the deleterious effects of palmitic acid and showing additional examples in specific tissues in text boxes might be a little redundant. As an alternative, we decided to describe the effects of oleic acid on the three main mechanisms responsible for palmitic acid-induced insulin resistance.

### **Comment 4. TEXT BOXES**

*I would suggest creating a text box instead of a figure for each of the tissues and transferring tissue-specific effects to these leaving common effects (S6K1, AMPK, etc.) in the main text. I would then suggest taking the current text box and incorporating it into the main text.*

Following the explanation provided in comment 3, we have modified the figures and we have decided to keep Text box I.

### **Comment 5. FIGURES AND FIGURE LEGENDS**

*The figures are good however a bit complicated to follow and a bit more complex than a typical TEM review figure.*

*Figure1: I would suggest keeping this figure however in order to make it a bit more visually accessible it might be useful to switch the location of the Insulin receptor and TLR4 so that the effector arrows do not cross over each other as much.*

*Figures2-5: as suggested above consider converting these to text boxes.*

*Figure1: perhaps incorporate these into the figures for palmitate or oleate respectively.*

We have simplified Figure 1. In addition, in the revised version of the figure the three main mechanisms activated by palmitic acid have been highlighted. We greatly appreciate the suggestion of the editor to make less complicated the figure.

Figures 2-4 have been modified and now reflect the preventive effects of oleic acid on the three main mechanisms by which palmitic acid promotes insulin resistance.

We have kept Figure1.

**Comment 6. LENGTH**

*At just over 4900 words the manuscript is significantly longer than we allow for a TEM review. Typically we allow between 3500 with some room for expansion. This means that the manuscript should be edited significantly as stated above.*

We have reduced the length of the manuscript to 4197 words.

**Comment 7. CLARITY/ACCESSIBILITY**

*Please review the changes I have suggested in the main text and make any modifications necessary if the intended scientific meaning has been altered.*

We have accepted the suggested changes.

## Comments for Reviewer 1

We would like to thank the reviewer for his/her useful suggestions, which have allowed us to include changes to improve the manuscript.

### Specific Comments:

**Comment 1.** *In page 8, the title next to "Oleic acid prevents palmitic acid-induced insulin resistance in metabolic tissues" seems not suitable to show "Adipose tissue". Please revise to easier way.*

Following the suggestion of the editor and reviewer 2, we have re-organized the manuscript and in the revised version we describe the preventive effects of oleic acid on the three main mechanisms activated by palmitic acid: 1. Increased synthesis of deleterious complex lipids. 2. Impaired function of cellular organelles. 3. Receptor mediated inflammation.

Therefore, in the new version of the manuscript this heading no longer exists.

**Comment 2.** *Skeletal muscle is a key metabolic tissue that affects the metabolic state of the whole organism, as you mentioned. But skeletal muscle was arranged not at the first section. Why?*

The reviewer is right and in the revised version of the manuscript the main effects of oleic acid on skeletal muscle are shown in the first section.

**Comment 3.** *Similar to the clear indications of palmitic acid-induced insulin resistance, it would be better to summarize the clear mechanisms for oleic acid prevents palmitic acid-induced insulin resistance.*

We believe that the re-organization of the manuscript has improved this aspect (please, see comment 1).

**Comment 4.** *Suitable dose for oleic acid or OEA and the damage dose of palmitic acid in human subjects were ignored. Why?.*

Usually, scientific associations make general assessments or recommendations about diet. For instance, the general recommendation for obesity and diabetes is to follow a low-fat diet (<30% of total energy). In addition, these scientific associations recommend replacing saturated with monounsaturated or polyunsaturated fat (please, see Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. *Circulation* 2017, in press; or reference 69 of the manuscript). In contrast, no doses are recommended.

**Comment 5.** *In Figure 2, receptors for oleic acid were indicated as GPCR but it was different in Figure 3, 4 and 5. Please give the reason(s) in context.*

These receptors have historically received two names. For instance, FFA receptor 1 (FFAR1) or G protein-coupled receptor 40 (GPR40). In the revised version of the manuscript we have included both names.

**Comment 6.** *GPR120 was not showed in Figure 2 for binding with oleic acid. Why?*

In the revised version of the manuscript this receptor appears in Figure 4 and it is bound to oleic acid.

**Comment 7.** *A high similarity (66 %) with previous report (McAinch AJ, Cornall LM, Watts R, et al., Eur J Nutr (Germany), 2015, 54(7): 1033-1043) observed. Please check it.*

We did not use this reference for preparing the manuscript that describes the effects of fatty acids on PDK4 in cultured myotubes from obese and diabetic individuals. Any similarity is by chance.

## Comments for Reviewer 2

We are very grateful to the comments of this reviewer (*The topic is very important, and writing a review which summarizes the current knowledge in this field would be highly appreciated by researchers. Such review is also important in the context of our efforts to prevent or to delay the disease.*).

In addition, we would like to thank the reviewer for her/his useful suggestions, which have allowed us to include changes to improve the manuscript.

### Major comments:

**Comment 1.** *I acknowledge the complexity of writing a review on this topic. However, I believe that the review can be improved if the text is slightly re-organized and presented in a somewhat different way. In the current form, the review is not easy to follow and also the focus and the aim of the review is kind of blurred. I think that figures are also not optimal and not really facilitate the reading. Authors prepared 5 figures with schematic views of how fatty acids affect cells. I assume that the first figure is supposed to give an overall view of palmitate effects on the cells whereas figures 2-5 should reflect the tissue-specific protection by oleate.*

*However, the impression from the text and from the figures is that the intention of authors is to compare the mechanisms of fatty acid action on different cells. The review makes readers to think that some mechanisms are tissue-specific. However, I do not think that these messages are what authors aim to say.*

The reviewer is right and we greatly appreciate his/her efforts to improve the manuscript. Following the suggestion of the reviewer and the editor, we have re-organized the manuscript. In the revised version of the manuscript we first describe the three main mechanisms by which palmitic acid increases insulin resistance (Figure 1) and then we describe the beneficial effects of oleic acid on these palmitic acid-activated mechanisms (Figures 2-4).

**Comment 2.** *Some specific comments that the major comment is based on (something is missing here)*

*Title of the first section is "Increased plasma non-esterified fatty acids link obesity and insulin resistance". However, in the text they claim that "Increased levels of NEFA provoke insulin resistance and impair  $\beta$ -cell function, making them good candidates as a link between obesity and T2DM development". With this sentence authors link NEFA with beta-cell dysfunction. I think an information about the toxic effect of NEFA on beta cells would be relevant and helpful in this section.*

The link between NEFA with beta-cell dysfunction is explained thoroughly later; please see page 10 (*Chronic exposure of rodent and human islet  $\beta$  cells to FA impairs glucose-stimulated insulin secretion (GSIS) and induces apoptosis*).

In our opinion, including information in this section about the toxic effects of NEFA on beta cells is a little bit premature. However, following the suggestion of the reviewer, we have included a reference of a review on the toxic effects of increased NEFA on beta cells to provide more information to the readers interested in this subject.

**Comment 3.** *In the second section "Molecular mechanisms involved in palmitic acid-induced insulin resistance" authors write that "Three main mechanisms have been reported*

*in palmitic acid-mediated insulin resistance and T2DM". However, it is not clear what authors mean by these three mechanisms. Those mechanisms need to be clarified at the beginning of the section. After reading of whole section my understanding is that these mechanisms are: 1) uptake of NEFA by non-adipose tissue, 2) impairment of cellular organelles, 3) activation of pro-inflammatory pathways through membrane receptors. Authors need to explain of how the effects of fatty acids have been classified into these three mechanisms. Especially, if ER stress and inflammation are also discussed in the first mechanism.*

Following the suggestion of the reviewer, the three mechanisms have been clarified at the beginning of the section: 1) Increased synthesis of deleterious complex lipids; 2) Impaired function of cellular organelles; 3) Receptor-mediated inflammation.

As the reviewer indicates, inflammation and ER stress are also mentioned in the first mechanism because some of these pathways intersect. However, the increased synthesis of ceramide and diacylglycerol provokes additional effects, independent of ER stress or inflammation, which also lead to insulin resistance. For example, diacylglycerol and ceramide have the capacity to activate PKC. This kinase phosphorylates IRS1 at serine residues, attenuating the insulin signaling pathway.

**Comment 4.** *Third section "Oleic acid prevents palmitic acid-induced insulin resistance in metabolic tissues" is short. Also, what is written in this section is already discussed in the first section. It is not really clear for the reader why it appears again.*

This paragraph has been rephrased and the subheading has been eliminated. We have kept this paragraph as the introduction for the next subheadings of the manuscript.

**Comment 5.** *Authors continue the paper by discussing the adipose tissue, skeletal muscle, liver and beta cells in separate sections. Such a design makes readers to think that the protective effect of oleate is tissue-specific. However, most mechanisms behind the toxic as well as protective effects of fatty acids are common for all tissues. For example, the role of GPR120 and inflammasome are discussed only in "adipose" section, which can make readers with no expertise in the field to think that this mechanism is adipose-specific.*

We believe that the re-organization of the revised version of the manuscript has solved this problem (please, see comment 1).

#### **Minor comments:**

**Comment 1.** *Some sentences can be formulated in a better way. Examples from page 3: "More than 90% of type 2 diabetic patients are overweight or obese, and obesity is associated with insulin resistance"; "Plasma NEFA arise from adipose tissue and the FA content of this tissue reflects fat intake"; "Although elevated plasma NEFA contribute to T2DM development, the saturated FA (SFA) (See Box 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differ significantly in their contribution to insulin resistance".*

Example 1: Following the suggestion of the editor, this sentence has been deleted in the revised version of the manuscript.

Example 2: This sentence has been substituted by "Plasma NEFA released by adipose tissue reflects fat intake".

Example 3: This sentence has been substituted by “However, the saturated FA (SFA) (Box 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differently contribute to insulin resistance”.

**Comment 2.** *In "adipose" section authors discuss about the diabetogenic effect of the tissue and the role of macrophages in this process but do not provide references.*

Following the suggestion of the reviewer, we have included two additional references in this paragraph:

. Bai Y, Sun Q. Macrophage recruitment in obese adipose tissue. *Obes Rev.* 2015 Feb;16(2):127-36.

. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL. Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? *Front Immunol.* 2014 Sep 26;5:470

**Comment 3.** *In the figures, palmitate uptake is shown to be mediated only by CD36 but this mechanism constitutes only 50% of the total uptake.*

The reviewer is right. In the revised version of the manuscript we have included FATP.